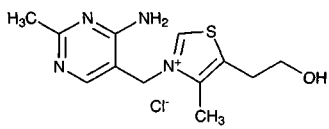
CHROMATOGRAM
Retention time: 2.6

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Thiamine



Molecular formula: $C_{12}H_{17}ClN_4OS$
Molecular weight: 300.81
CAS Registry No.: 59-43-8, 67-03-8 (HCl), 532-43-4 (mononitrate)
Merck Index: 9430

SAMPLE
Matrix: blood
Sample preparation: 1 mL Hemolysate + 30 μL 4 μM IS in 100mM HCl, shake thoroughly. Slowly add 2 mL MeOH, mix, let stand for 30 min. Centrifuge at 2000 g for 10 min. Add 50 μL freshly prepared 30.4 mM potassium ferricyanide and 50 μL 0.8 mM NaOH to 1 mL supernatant. Filter (0.45 μm) and inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4.0 Spherisorb NH2
Column: 125 × 4.0 5 μm Spherisorb NH2
Mobile phase: MeOH:100 mM pH 7.5 potassium phosphate buffer 45:55
Flow rate: 1
Injection volume: 50
Detector: F ex 375 em 430

CHROMATOGRAM
Retention time: 4.0
Internal standard: acetylaneurine (3.0)
Limit of detection: 2 nM

KEY WORDS
erythrocytes

REFERENCE
Lynch,P.L.M.; Trimble,E.R.; Young,I.S. High-performance liquid chromatographic determination of thiamine diphosphate in erythrocytes using internal standard methodology, *J.Chromatogr.B*, **1997**, *701*, 120–123.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma, whole blood, or erythrocytes + 200 μL 100 mg/mL trichloroacetic acid, vortex vigorously, centrifuge at 35000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 μBondapak C18
Mobile phase: MeCN:buffer 3.8:96.2 (Mobile phase was 200 mM NaH_2PO_4 in 3 g/L MeCN in water.)
Flow rate: 1
Injection volume: 100
Detector: F ex 375 em 450 following post-column reaction. The column effluent mixed with the reagent pumped at 0.5 mL/min and flowed to the detector. (Reagent was 100 μg/mL potassium ferricyanide in 150 g/L NaOH.)

CHROMATOGRAM
Retention time: 8.0 (thiamine), 3.1 (thiamine triphosphate), 3.8 (thiamine pyrophosphate), 5.0 (thiamine monophosphate)
Limit of detection: 30 fmole

KEY WORDS
post-column reaction; pharmacokinetics; plasma; whole blood; erythrocytes

REFERENCE
Kimura,M.; Itokawa,Y. Determination of thiamin and thiamin phosphate esters in blood by liquid chromatography with post-column derivatization, *Clin.Chem.*, **1983**, *29*, 2073–2075.

SAMPLE
Matrix: blood
Sample preparation: Hemolyze whole blood by freezing at -20° for 20 min, thaw, homogenize. Add a 200 μL aliquot of hemolyzed blood or serum to 200 μL chilled 10% perchloric acid, let stand below 4° for 15 min, centrifuge at 10000 g for 1 min. Remove a 200 μL aliquot of the supernatant and add it to 1.8 M sodium acetate containing 600 mM NaOH, mix, filter (Costar filter unit) while centrifuging for 30 s, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: present but not specified
Column: 110 × 4.7 Partisphere 5 C18 (Whatman)
Mobile phase: Gradient. A was 15 mM citric acid adjusted to pH 4.2 with 50% ammonium hydroxide, prepare fresh each day. B was 100 mM formic acid containing 4% diethylamine, pH

3.2. A:B from 90:10 to 50:50 over 2.5 min, to 5:95 over 0.5 min, maintain at 5:95 over 3.5 min, to 95:5 over 0.5 min, maintain at 95:5 for 3 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 365 em 435 following post-column reaction. The column effluent mixed with the reagent pumped at 0.2 mL/min and the mixture flowed through a 90 cm × 0.5 mm ID PTFE coil to the detector. (Prepare reagent by dissolving 100 mg potassium ferricyanide in 120 mL 3 M NaOH.)

CHROMATOGRAM
Retention time: 7.6
Limit of quantitation: 2 nM

OTHER SUBSTANCES
Extracted: thiamine monophosphate, thiamine pyrophosphate, thiamine triphosphate

KEY WORDS
post-column reaction; whole blood; serum

REFERENCE
Lee,B.L.; Ong,H.Y.; Ong,C.N. Determination of thiamine and its phosphate esters by gradient-elution high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *567*, 71–80.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 150 μL 3 M perchloric acid, vortex, centrifuge at >1500 g. Remove 500 μL of the supernatant and add it to 300 μL 1 M pH 4.6 acetate buffer, add 100 μL 10 mg/mL acidic phosphatase (2 U/mg, grade II, Boehringer Mannheim) in water, heat at 40° for 16 h, add 150 μL 3 M perchloric acid, vortex, centrifuge at >1500 g, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 125 × 4 Nucleosil 120 5 C18
Mobile phase: MeCN:buffer 25:75 (Buffer was 10 mM perchloric acid containing 10 mM octanesulfonic acid.)
Flow rate: 2
Injection volume: 20
Detector: F ex 365 em 435 following post-column reaction. The column effluent mixed with 0.8 g/L potassium ferricyanide in 3 M NaOH pumped at 1 mL/min, the mixture flowed through a 10 m × 0.3 mm i.d. PTFE coil at 30° to the detector.

CHROMATOGRAM
Retention time: 2.3
Limit of detection: 2 ng/mL

KEY WORDS
post-column reaction; plasma; pharmacokinetics

REFERENCE
Mascher,H.; Kikuta,C. High-performance liquid chromatographic determination of total thiamine in human plasma for oral bioavailability studies, *J.Pharm.Sci.*, **1993**, *82*, 56–59.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation.

Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 2.597

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: feed
Sample preparation: 1 g Ground feed + 20 mL 100 mM HCl, shake vigorously, heat on a boiling water bath for 30 min (shake every 5 min), cool in an ice bath for 5 min, centrifuge at 1000 rpm for 10 min. Remove a 5 mL aliquot and make it up to 50 mL with buffer, centrifuge at 1000 rpm for 5 min, inject a 10 μL aliquot. (Buffer was water adjusted to pH 4.0 with acetic acid.)

HPLC VARIABLES
Column: 250 × 4.6 SynChropack SCD-100 (SynChrome Inc.)
Mobile phase: MeOH:water 40:60 containing 50 mM sodium pentanesulfonate, pH adjusted to 4.0 with acetic acid
Flow rate: 1.5
Injection volume: 10
Detector: F ex 370 em 430 following post-column derivatization. The column effluent was mixed with 200 mM KOH and 0.01% potassium ferricyanide, each pumped at 0.5 mL/min. The mixture flowed in the dark through a 3 m × 0.8 mm i.d. knotted coil of PTFE tubing to the detector.

CHROMATOGRAM
Retention time: 5
Limit of detection: 5 pg

KEY WORDS
post-column reaction; derivatization

REFERENCE
Gehring,T.A.; Cooper,W.M.; Holder,C.L.; Thompson,H.C.,Jr. Liquid chromatographic determination of thiamine in rodent feed by postcolumn derivatization and fluorescence detection, *J.AOAC Int.*, **1995**, *78*, 307–309.

SAMPLE
Matrix: food
Sample preparation: Condition a CBA (methylcarboxylate in acid form) SPE cartridge with 1 mL MeOH and 1 mL 10 mM pH 4.0 phosphate buffer. Add a sample of finely-ground food to 20 mL 100 mM HCl and heat at 100° for 30 min. Cool and adjust pH to 4.4-4.5 with sodium acetate. Add a 6 mg/mL solution of takadiastase (Fluka). Heat at 47° for 3 h. Filter through a

cellulose acetate filter and dilute with water to 50 mL. Add a 2 mL aliquot to the SPE cartridge. Wash twice with 500 μL portions of pH 4.0 phosphate buffer. Elute with three 200 μL portions of 100 mM barium chloride. Inject an aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 100RP-18
Mobile phase: MeOH:10 mM pH 2.8 phosphate buffer:triethylamine 25:85:0.1 containing 5 mM hexanesulfonic acid
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 6.25
Limit of detection: 408 ng/g

KEY WORDS
baby food; cereal; dietetic cookies; SPE

REFERENCE
Blanco,D.; Llaneza,M.B.; Gutierrez,M.D. A paired ion liquid chromatographic method for thiamine determination in selected foods, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2155–2164.

SAMPLE
Matrix: formula, milk
Sample preparation: Mix 8.0 g powdered infant milk with 10 mL water to it. Mix the diluted powder or 10.5 g liquid infant milk with 1 g solid trichloroacetic acid, shake thoroughly with magnetic stirring for 10 min, centrifuge at 1250 g for 10 min, add 3 mL 4% trichloroacetic acid to the solid residue, mix thoroughly for 10 min, centrifuge, discard the solid phase. Combine the two acid extracts and make up to 10 mL with 4% trichloroacetic acid, filter (0.45 μm), inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Column: 250 × 4.6 5 μm Tracer Spherisorb ODS 2 C18 (Teknokroma, Spain)
Mobile phase: MeOH:buffer 15:85 (Buffer was 5 mM octanesulfonic acid and 0.5% triethylamine, pH 3.6.)
Flow rate: 1
Injection volume: 20
Detector: UV 261 for 6 min, UV 287 for 2 min, UV 290 for 5 min, UV 282 for 3 min, UV 268 for 3.5 min, UV 361 for 20.5 min, UV 246 for 20 min

CHROMATOGRAM
Retention time: 48
Limit of quantitation: ≤100 ng/mL

OTHER SUBSTANCES
Extracted: riboflavin, pyridoxine, vitamin B12, folic acid, niacinamide, pyridoxal, pyridoxamine

REFERENCE
Albal-Hurtado,S.; Veciana-Nogués,M.; Izquierdo-Pulido,M.; Mariné-Font,A. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *778*, 247–253.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate 75 mg powdered tablets with 25 mL mobile phase for 15 min, filter (paper), inject a 135 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 (Scharlau Science, Spain)
Mobile phase: MeOH:20 mM KH_2PO_4 30:70 adjusted to pH 4.0 with orthophosphoric acid

Flow rate: 1.5
Injection volume: 135
Detector: UV 246

CHROMATOGRAM
Retention time: 2.3
Limit of quantitation: 1.9 μg/mL

OTHER SUBSTANCES
Simultaneous: aspirin, caffeine, salicylic acid

KEY WORDS
tablets

REFERENCE
Gámiz-Gracia,L.; Luque de Castro,M.D. An HPLC method for the determination of vitamin B1, caffeine, acetylsalicylic acid, and the impurities of salicylic acid in a pharmaceutical preparation, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2123–2133.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets if necessary. Add tablets to 100 mL 5 mM pH 4.5 potassium phosphate buffer, sonicate at 75 W for 2 min, cool to room temperature, make up to 200 mL with buffer, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 10 μm LiChrosorb NH2 aminopropyl
Mobile phase: MeCN:5 mM KH_2PO_4 87:13 (Wash column with MeCN:water 10:90 at the end of the day.)
Column temperature: 25
Flow rate: 2
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 3.5

OTHER SUBSTANCES
Simultaneous: pantothenic acid, riboflavin, niacinamide, pyridoxine

KEY WORDS
tablets

REFERENCE
Hudson,T.J.; Allen,R.J. Determination of pantothenic acid in multivitamin pharmaceutical preparations by reverse-phase high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 113–115.

SAMPLE
Matrix: formulations
Sample preparation: Tablets without iron. Grind 5 tablets to a fine powder, add 10 mL monothioglycerol and 800 mL buffer, sonicate for 30 min, add 150 mL MeOH, make up to 1 L with buffer, filter (GF/C paper), discard first few mL, remove a 10 mL aliquot, make up to 25 mL with mobile phase, inject an aliquot. Tablets with dioctyl sodium sulfosuccinate. Grind 5 tablets to a fine powder, add 10 mL 2-monothioglycerol and 1 g barium chloride, make up to 1 L with buffer, stir vigorously for 30 min, filter (GF/C paper), discard first few mL, inject an aliquot. Capsules with iron. Contents of one capsule + 5 mL 2-monothioglycerol + 2 mL glacial acetic acid + 75 mL buffer, sonicate for 5 min, make up to 100 mL with buffer, stir vigorously for 30 min, filter (GF/C paper), add 300 mg cupferron, stir for 10 min, let stand for 1 h at room temperature, filter (GF/C paper), let stand for 30 min, filter again (if necessary), discard first few mL, inject an aliquot. (Buffer was 48 mL glacial acetic acid and 10 mL triethylamine in 1

L water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine, make up to 1.7 L with water.)

HPLC VARIABLES
Column: 100 × 8 Radial Pak A C18 (Waters)
Mobile phase: MeOH:buffer 15:85 (Buffer was 2.20 g sodium heptanesulfonate, 100 mg EDTA, 48 mL glacial acetic acid, and 10 mL triethylamine made up to 1.7 L with water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine.)
Flow rate: 2
Injection volume: 10
Detector: UV 280

CHROMATOGRAM
Retention time: 9

OTHER SUBSTANCES
Simultaneous: niacinamide, riboflavin, pyridoxine, ascorbic acid (UV 254)

KEY WORDS
multi-vitamin; protect from light; tablets; capsules

REFERENCE
Lam,F.-L.; Holcomb,I.J.; Fusari,S.A. Liquid chromatographic assay of ascorbic acid, niacinamide, pyridoxine, thiamine, and riboflavin in multivitamin-mineral preparations, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 1007–1011.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES
Guard column: pellicular Corasil
Column: 10 μm μBondapak C18
Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B 100:0 for 19 min then 0:100 (step gradient) or A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 31 (step gradient), 24 (curve gradient)

OTHER SUBSTANCES
Simultaneous: folic acid (UV 280), niacin, niacinamide, pyridoxamine (UV 280), riboflavin, pyridoxine (UV 280), ascorbic acid (UV 280)

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 500 mg ground tablets, extract with water, make up to 50 or 100 mL with water, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 10 C18
Mobile phase: MeOH:1% acetic acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: menadione hydrogen sulfite, niacinamide, pyridoxine, riboflavin, ascorbic acid

KEY WORDS
tablets; multi-vitamin

REFERENCE
Sadlej-Sosnowska,N.; Blitek,D.; Wilczynska-Wojtulewicz,I. Determination of menadione sodium hydrogen sulphite and nicotinamide in multivitamin formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *357*, 227–232.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, pantothenic acid, pyridoxine, riboflavin, saccharin, vitamin B12, ascorbic acid

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, **1993**.

SAMPLE
Matrix: formulations
Sample preparation: Dilute liquid multivitamin formulations, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Lichrosorb RP-8
Mobile phase: Gradient. A was 10 mM KH_2PO_4 containing 5 mM sodium hexanesulfonate adjusted to pH 2.8 with phosphoric acid. B was MeOH. A:B from 90:10 to 71.8:28.2 over 4 min, maintain at 71.8:28.2 for 1.5 min, to 50:50 over 6.5 min, maintain at 50:50 for 5 min, return to initial conditions over 5 min
Flow rate: 1

Injection volume: 5
Detector: UV 272

CHROMATOGRAM
Retention time: 9.90
Internal standard: theobromine (8)
Limit of detection: 0.430 ng

OTHER SUBSTANCES
Simultaneous: folic acid, niacin, niacinamide, riboflavin, pyridoxine (UV 290)

KEY WORDS
liquid multivitamins; degas solutions with helium; protect from light

REFERENCE
Blanco,D.; Sánchez,L.A.; Gutiérrez,M.D. Determination of water soluble vitamins by liquid chromatography with ordinary and narrow-bore columns, *J.Liq.Chromatogr.*, **1994**, *17*, 1525–1539.

SAMPLE
Matrix: rice
Sample preparation: Heat ground rice with at least 10 volumes of 100 mM HCl at 95-100° for 30 min with frequent mixing, cool, dilute to a thiamine concentration of 200 ng/mL with 100 mM HCl, adjust the pH of a 65 mL aliquot to 4.0-4.5 with about 5 mL 2 M sodium acetate, add 5 mL 10% takadiastase in water, heat at 45-50° for 3 h, adjust pH to 3.5, make up to 100 mL with water, filter (paper) (AOAC Official Methods of Analysis, 1990, 1049), centrifuge at 3500 rpm for 20 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4 Nucleosil 5 C18
Mobile phase: 10 mM NaH_2PO_4 containing 150 mM sodium perchlorate, adjusted to pH 2.2 with perchloric acid
Column temperature: 55
Flow rate: 0.6
Injection volume: 25
Detector: F ex 375 em 435 following post-column reaction. The column effluent mixed with 0.1% potassium hexacyanoferrate(III) in 12% NaOH pumped at 0.6 mL/min and this mixture flowed through a 30 cm × 0.8 mm ID stainless steel coil at 55° to the detector.

CHROMATOGRAM
Retention time: 8.5

KEY WORDS
post-column reaction

REFERENCE
Ohta,H.; Baba,T.; Suzuki,Y.; Okada,E. High-performance liquid chromatographic analysis of thiamine in rice flour with fluorimetric post-column derivatization, *J.Chromatogr.*, **1984**, *284*, 281–284.

SAMPLE
Matrix: rice
Sample preparation: Grind rice to pass 30-mesh screen. Stir 3 g ground rice and 50 mL MeOH: 100 mM HCl 40:60 with a glass rod until homogeneous, reflux for 30 min, vortex for 1 min, sonicate for 20 min, centrifuge at 3000 g for 20 min, filter (0.45 μm) the supernatant, inject a 5 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax TMS
Mobile phase: 10 mM NaH_2PO_4 containing 500 mM sodium perchlorate adjusted to pH 2.5 with 3 M perchloric acid
Column temperature: 55
Flow rate: 0.4

Injection volume: 5
Detector: F ex 375 em 435 following post-column reaction. The column effluent mixed with reagent pumped at 0.4 mL/min, the mixture flowed through a 300 × 0.8 stainless steel mixing coil to the detector. (Reagent was 0.1% potassium hexacyanoferrate(III) in 15% NaOH.)

CHROMATOGRAM
Retention time: 8
Limit of quantitation: 1.5 μg/g

KEY WORDS
post-column reaction

REFERENCE
Ohta,H.; Maeda,M.; Nogata,Y.; Yoza,K.-I.; Takeda,Y.; Osajima,Y. A simple determination of thiamine in rice (*Oryza sativa* L.) by high-performance liquid chromatography with post-column derivatization, *J.Liq.Chromatogr.*, **1993**, *16*, 2617–2629.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Accubond Amino (J & W)
Mobile phase: MeCN:buffer 10:90 (Buffer was 20 mM phosphoric acid adjusted to pH 3.0 with 20 mM NaOH.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 1.2

OTHER SUBSTANCES
Simultaneous: p-aminobenzoic acid, niacinamide, pyridoxal, pyridoxamine, riboflavin, pyridoxine, vitamin B12

REFERENCE
J & W Catalog, 1992-3, p. 277.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 15:85 (Buffer was 4.3 mM sodium hexanesulfonate containing 0.1% triethylamine, adjusted to pH 2.8 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Detector: UV 200

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Simultaneous: niacin, pantothenic acid, pyridoxine, riboflavin, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994**, p. 780.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 100 × 4.6 Spheri-5 RP-8
Mobile phase: Gradient. A was 100 mM pH 4.7 acetate buffer. B was MeCN:100 mM pH 4.7 acetate buffer 25:75.
Column temperature: 26
Flow rate: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Simultaneous: niacin, pyridoxine, riboflavin, niacinamide, ascorbic acid

REFERENCE
Rainin Catalog, C1-94, **1994,** p. 7.21.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, predni-

solone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize (motor-driven glass homogenizer) 100 mg tissue with 300 μL 5% trichloroacetic acid at 4°, centrifuge at 4° at 5000 g for 1 h. Wash the supernatant with 3 volumes of water-saturated diethyl ether for 1 h. Remove an 80 μL aliquot of the aqueous phase and add it to 50 μL reagent, mix for 5-10 s, inject an aliquot 1 min after the addition of the reagent. (Prepare reagent by mixing 50 μL 10 mg/mL potassium ferricyanide with 2.5 mL 15% NaOH, store in the dark, discard after 1 day.)

HPLC VARIABLES

Guard column: 4.2 × 3.2 30-44 μm 201RP (Vydac)
Column: 150 or 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. MeOH:25 mM pH 8.4 phosphate buffer 10:90 for 1 min, to 100:0 over 3 min, maintain at 100:0 for 2 min, return to initial conditions over 2 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 390 em 475

CHROMATOGRAM

Retention time: 4.2 (thiamine triphosphate), 4.5 (thiamine pyrophosphate), 5 (thiamine monophosphate), 6 (thiamine)
Limit of detection: 0.05 pmole

KEY WORDS

derivatization; rat; nerve; heart

REFERENCE

Bontemps,J.; Philippe,P.; Bettendorff,L.; Lombet,J.; Dandrifosse,G.; Schoffeniels,E.; Crommen,J. Determination of thiamine and thiamine phosphates in excitable tissues as thiochrome derivatives by reversed-phase high-performance liquid chromatography on octadecyl silica, *J.Chromatogr.*, **1984**, *307*, 283–294.

SAMPLE

Matrix: tissue

Sample preparation: Condition a 500 mg Nucleosil C18 SPE cartridge with 2 mL MeOH, 2 mL MeOH containing 5 mM sodium heptanesulfonate, and two 2 mL portions of water. Suspend 5 g homogenized tissue with 35 mL 10 mM HCl, autoclave at 121° for 30 min, add 2 mL 25 mg/mL taka-distase (Fluka) in 2.5 M sodium acetate, add 2 mL 10 (muscle) or 20 (liver) mg/mL clara-distase (Fluka) in water, add 2 mL 50 mg/mL papain (Merck) in water, adjust pH to 4.5, heat at 37° for 16-18 h, filter (paper), adjust pH to 6.5, filter again, make up to 50 mL with water, add 4 mL to the SPE cartridge, wash with 2 mL MeOH:water 20:80 containing 5 mM sodium heptanesulfonate, elute with 2 mL MeOH:water 50:50 containing 5 mM sodium heptanesulfonate, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 10 μm Nucleosil C18
Column: 150 × 4.6 3 μm Nucleosil C18

Mobile phase: MeCN:10 mM pH 3.0 KH_2PO_4 16:84 (muscle) or 15:85 (liver) containing 5 mM sodium heptane sulfonate (Wash with MeCN:water 20:80 at the end of the day, store column in MeCN.)
Column temperature: 45
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 6.5 (muscle), 8 (liver)
Limit of detection: 500 ng/g

OTHER SUBSTANCES
Extracted: riboflavin

KEY WORDS
pig; muscle; liver; protect from light; SPE

REFERENCE
Barna,I.; Dworschák,E. Determination of thiamine (vitamin B1) and riboflavin (vitamin B2) in meat and liver by high-performance liquid chromatography, *J.Chromatogr.A*, **1994**, *668*, 359–363.

SAMPLE
Matrix: yeast
Sample preparation: Condition CM-cellulose (44-105 μm CM-Cellulofine C-200, Biochemical Industry, Tokyo) by washing with 200 mM HCl, 200 mM NaOH, water, and 200 mM HCl, rinse with water until the rinses are neutral. Add a 2.5 mL aliquot to a 170 × 10 column, wash with 300 mM phosphoric acid, wash with water until the eluate is neutral. Heat 1 g dried yeast, 1 mL 10% HCl, and 80 mL water with frequent shaking at 80-85° for 30 min, cool, make up to 100 mL with water, centrifuge for 10 min. Remove a 4 mL aliquot of the supernatant and add it to 5 mL 200 mM pH 4.5 acetic acid/sodium acetate buffer and 1 mL 30 mg/mL Taka-diastase supernatant (Sankyo) in 5 mM HCl, heat at 45-50° for 3 h, add 2 mL of this mixture to the column at 0.5 mL/min, wash with two 10 mL portions of water at 1 mL/min, elute with two 2.5 mL portions of 300 mM phosphoric acid at 0.5 mL/min. Add 1 mL 1 μg/mL IS and 10 mg sodium 1-octanesulfonate to the eluate, mix, inject a 200 μL aliquot.

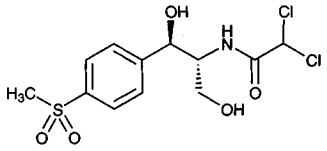
HPLC VARIABLES
Column: 150 × 4.6 Capcell-pak C18 (Shiseido)
Mobile phase: MeCN:buffer 20:80 (Buffer was 20 mM pH 3.5 phosphate buffer containing 0.2% sodium 1-octanesulfonate.)
Column temperature: 40
Flow rate: 1
Injection volume: 200
Detector: UV 254

CHROMATOGRAM
Retention time: 7.5
Internal standard: phenacetin (10)
Limit of quantitation: 250 ng/mL

KEY WORDS
SPE

REFERENCE
Yamanaka,K.; Matsuoka,M.; Banno,K. Determination of thiamine in dried yeast by high-performance liquid chromatography using a clean-up column of CM-cellulose, *J.Chromatogr.A*, **1996**, *726*, 237–240.

Thiamphenicol



Molecular formula: $C_{12}H_{15}Cl2NO_5S$
Molecular weight: 356.23
CAS Registry No.: 15318-45-3
Merck Index: 9436
Lednicer No.: 2 45

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 7

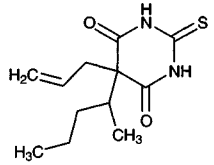
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Thiamylal



Molecular formula: $C_{12}H_{18}N_2O_2S$
Molecular weight: 254.35
CAS Registry No.: 77-27-0, 337-47-3 (Na salt)
Merck Index: 9437
Lednicer No.: 1 274

SAMPLE

Matrix: bile, blood, gastric contents, tissue, urine
Sample preparation: Homogenize tissue with four volumes 100 mM pH 5 phthalate buffer. 2 mL Blood, bile, urine, stomach contents, or tissue homogenate + 2 mL 100 mM pH 5 phthalate buffer + 100 μL 1 mg/mL phenolphthalein in MeOH, vortex for 10 s, add to a Clin Elut SPE

cartridge (Analytichem), let stand for 10 min, elute with three 5 mL portions of dichloromethane. Evaporate the eluate to dryness under a stream of air at 60°, reconstitute with 200 μL MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS (Altex)
Mobile phase: MeOH:water 5:1
Flow rate: 2
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Internal standard: phenolphthalein

KEY WORDS
SPE; liver; kidney

REFERENCE
Costantino,A.G.; Caplan,Y.H.; Levine,B.S.; Dixon,A.M.; Smialek,J.E. Thiamylal: review of the literature and report of a suicide, *J.Forensic Sci.*, **1990**, *35*, 89–96.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL MeCN, vortex for 10 s, let stand for 10 min, vortex for 10 s, centrifuge at 12000 g for 2 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 6 5 μm Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeCN:water 55:45
Flow rate: 1.2
Injection volume: 20
Detector: UV 288

CHROMATOGRAM
Retention time: 4.9
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: thiopental
Noninterfering: acetaminophen, allopurinol, amikacin, amobarbital, amphotericin B, ampicillin, aspirin, barbital, caffeine, carbenicillin, chloramphenicol, chlorpromazine, cimetidine, cisplatin, cyclophosphamide, cyclosporin A, cytarabine, dactinomycin, doxorubicin, droperidol, ethosuximide, 5-fluorocytosine, 5-fluorouracil, furosemide, gentamicin, hexobarbital, ketamine, ketoconazole, 6-mercaptopurine, metharbital, methotrexate, miconazole, mizoribine, pentobarbital, phenobarbital, procainamide, secobarbital, tegafur, vancomycin

KEY WORDS
pharmacokinetics; plasma

REFERENCE
Hosotsubo,H.; Takeda,K.; Hosotsubo,K.; Yoshiya,I. Measurement of thiamylal in human plasma using reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *487*, 204–209.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum or plasma + 100 μL MeOH + 1 mL 70 mM pH 6.4 phosphate buffer + 5 mL n-pentane, shake vigorously for 10 min, centrifuge at 1500 g for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 200 μL mobile phase, vortex for 1 min, inject a 5 μL aliquot.

HPLC VARIABLES
Column: Nucleosil 5 C8
Mobile phase: MeCN:buffer 35:65 (Buffer was 0.2 mM phosphoric acid containing 0.175 mM KH_2PO_4.)
Flow rate: 1.2
Injection volume: 5
Detector: UV 290

CHROMATOGRAM
Retention time: 12
Internal standard: thiamylal

OTHER SUBSTANCES
Extracted: thiopental

KEY WORDS
serum; plasma; cow; human; comparison with capillary electrophoresis; thiamylal is IS

REFERENCE
Meier,P.; Thormann,W. Determination of thiopental in human serum and plasma by high-performance capillary electrophoresis-micellar electrokinetic chromatography, *J.Chromatogr.*, **1991**, *559*, 505–513.

SAMPLE
Matrix: blood
Sample preparation: Mix serum with an equal volume of 1 M pH 5.0 phosphate buffer, add IS, add 3 mL ethyl acetate, rotate for 10 min, centrifuge at 3500 rpm for 5 min. Remove 2 mL of the organic layer and evaporate it to dryness, reconstitute the residue in 200 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shim-pack CLC-ODS (Shimadzu)
Mobile phase: MeOH:10 mM pH 5.0 sodium phosphate buffer 60:40
Flow rate: 1
Injection volume: 10-20
Detector: UV

CHROMATOGRAM
Internal standard: 5-(p-methylphenyl)-5-phenylhydantoin
Limit of quantitation: 1 μg/mL

KEY WORDS
serum; rat; pharmacokinetics

REFERENCE
Nakashima,E.; Matsushita,R.; Ohshima,T.; Tsuji,A.; Ichimura,F. Quantitative relationship between structure and peritoneal membrane transport based on physiological pharmacokinetic concepts for acidic drugs, *Drug Metab.Dispos.*, **1995**, *23*, 1220–1224.

SAMPLE
Matrix: blood
Sample preparation: Condition a 1 mL 50 mg Bond Elut C18 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of 10 mM KH_2PO_4. 100 μL Serum + 50 μL 25 μg/mL n-propyl p-hydroxybenzoate in water + 500 μL 10 mM KH_2PO_4, add to the SPE cartridge, wash with two 1 mL portions of water, elute with 300 μL MeOH. Evaporate the eluate, reconstitute in 100 μL EtOH:water 50:50, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 TSK-gel ODS 80TM
Mobile phase: EtOH:10 mM KH_2PO_4 15:85 containing 17 mM β-cyclodextrin (Suspend 17 mmoles β-cyclodextrin in 150 mL EtOH, make up to 1 L with 10 mM KH_2PO_4.)
Column temperature: 25

Flow rate: 0.9
Injection volume: 50
Detector: UV 288

CHROMATOGRAM
Retention time: 37 (S(-)), 39 (R(+))
Internal standard: n-propyl p-hydroxybenzoate (25)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: muscle relaxants, benzodiazepines

KEY WORDS
serum; chiral; SPE

REFERENCE
Sueyasu,M.; Ikeda,T.; Otsubo,K.; Taniyama,T.; Aoyama,T.; Oishi,R. Enantioselective determination of thiamylal in human serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *665*, 133–137.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 5 μm C-18 (Perkin-Elmer)
Mobile phase: MeOH:THF:buffer 50:7:43 (Buffer was prepared from equal volumes of 22 g/L KH_2PO_4 and 18 g/L Na_2HPO_4, pH 6.6 ± 0.2.)
Flow rate: 2
Injection volume: 6
Detector: UV 240

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetazolamide, amobarbital, aspirin, barbital, butabarbital, cefazolin, chlorothiazide, chlorzoxazone, dicloxacillin, ethosuximide, furosemide, hydrochlorothiazide, ibuprofen, oxacillin, pentobarbital, phenobarbital, phenylbutazone, phenytoin, salicylic acid, secobarbital, sulfamethoxazole, theophylline, thiopental, ascorbic acid
Noninterfering: ampicillin, penicillin G, valproic acid

REFERENCE
Kelner,M.; Bailey,D.N. Reversed-phase liquid-chromatographic simultaneous analysis for thiopental and pentobarbital in serum, *Clin.Chem.*, **1983**, *29*, 1097–1100.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN:water 25:75, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18 (Alltech)
Mobile phase: MeCN:4 mM potassium phosphate buffer 50:50, pH 4.0
Flow rate: 1.2
Injection volume: 100
Detector: UV 290

CHROMATOGRAM
Retention time: 6.9

OTHER SUBSTANCES
Simultaneous: amobarbital, heptabarbital, hexobarbital, methohexital, pentobarbital, phenobarbital, secobarbital, thiopental

Flow rate: 0.9
Injection volume: 50
Detector: UV 288

CHROMATOGRAM
Retention time: 37 (S(-)), 39 (R(+))
Internal standard: n-propyl p-hydroxybenzoate (25)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: muscle relaxants, benzodiazepines

KEY WORDS
serum; chiral; SPE

REFERENCE
Sueyasu,M.; Ikeda,T.; Otsubo,K.; Taniyama,T.; Aoyama,T.; Oishi,R. Enantioselective determination of thiamylal in human serum by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *665*, 133–137.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 125 × 4.6 5 μm C-18 (Perkin-Elmer)
Mobile phase: MeOH:THF:buffer 50:7:43 (Buffer was prepared from equal volumes of 22 g/L KH_2PO_4 and 18 g/L Na_2HPO_4, pH 6.6 ± 0.2.)
Flow rate: 2
Injection volume: 6
Detector: UV 240

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: acetaminophen, acetazolamide, amobarbital, aspirin, barbital, butabarbital, cefazolin, chlorothiazide, chlorzoxazone, dicloxacillin, ethosuximide, furosemide, hydrochlorothiazide, ibuprofen, oxacillin, pentobarbital, phenobarbital, phenylbutazone, phenytoin, salicylic acid, secobarbital, sulfamethoxazole, theophylline, thiopental, ascorbic acid
Noninterfering: ampicillin, penicillin G, valproic acid

REFERENCE
Kelner,M.; Bailey,D.N. Reversed-phase liquid-chromatographic simultaneous analysis for thiopental and pentobarbital in serum, *Clin.Chem.*, **1983**, *29*, 1097–1100.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN:water 25:75, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm C18 (Alltech)
Mobile phase: MeCN:4 mM potassium phosphate buffer 50:50, pH 4.0
Flow rate: 1.2
Injection volume: 100
Detector: UV 290

CHROMATOGRAM
Retention time: 6.9

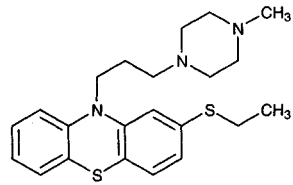
OTHER SUBSTANCES
Simultaneous: amobarbital, heptabarbital, hexobarbital, methohexital, pentobarbital, phenobarbital, secobarbital, thiopental

mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Thiethylperazine



Molecular formula: $C_{22}H_{29}N_3S_2$
Molecular weight: 399.62
CAS Registry No.: 1420-55-9, 52239-63-1 (maleate)
Merck Index: 9449

SAMPLE

Matrix: blood

Sample preparation: 3 mL Serum + 3 mL diluted Titrisol (pH 10 borate buffer, Merck) + 4 mL heptane:isoamyl alcohol 97:3, shake thoroughly for 15 s, centrifuge at 2500 g for 5 min. Remove the organic phase and add it to 1.5 mL 50 mM sulfuric acid containing 0.1% $Na_2S_2O_2$, mix for 15 s, centrifuge at 2500 g for 10 min. Remove the aqueous phase. Repeat the extraction and back extraction. Combine the aqueous phases and add them to 1.5 mL 2 M pH 9.1 glycine buffer, add 200 µL n-hexane:isoamyl alcohol 97:3, vortex for 25 s. Remove the organic phase and evaporate it to dryness in a desiccator, reconstitute in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 µm µBondapak C18
Mobile phase: MeCN:water:acetic acid 65:35:3 containing 10 mM dodecyl hydrogen sulfate
Flow rate: 2
Detector: UV 257

CHROMATOGRAM

Retention time: 13
Internal standard: thiethylperazine

OTHER SUBSTANCES

Extracted: perphenazine

Noninterfering: alimemazine, biperidine, carbamazepine, chlorpromazine, clomipramine, diazepam, dihydroergotamine, disulfiram, dixyrazine, haloperidol, levomepromazine, nitrazepam, orphenadrine, promethazine, propiomazine, thioridazine, trimipramine, vitamins

KEY WORDS
serum; thiethylperazine is IS

REFERENCE
Larsson,M.; Forsman,A. A high-performance liquid chromatographic method for the assay of perphenazine and its dealkylated metabolite in serum after therapeutic doses, *Ther.Drug Monit.*, **1983**, *5*, 225–228.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 3 5 μm Lichrosorb SI60
Mobile phase: MeCN:MeOH:ammonium hydroxide 250:55:13
Flow rate: 1.2
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 3.4

OTHER SUBSTANCES
Simultaneous: N-acetylprocainamide, amitriptyline, chlorimipramine, chlorpromazine, codeine, desipramine, dimethacrine, diphenhydramine, disopyramide, doxepin, hydroquinidine, maprotiline, melitracene, mesoridazine, nortriptyline, opipramol, perazine, perphenazine, procainamide, prochlorperazine, promazine, prothipendyl, protriptyline, thioperazine, thioridazine, trifluoperazine
Noninterfering: acenocoumaron, acetaminophen, acetophenetidine, aspirin, benzodiazepines, bibenzepin, butriptyline, caffeine, chlorprothixene, clopenthixol, clothiapine, dixyrazine, droperidol, fluphenazine, haloperidol, hydroxyzine, isoniazid, methotrimeprazine, metopimazine, moperone, noxiptyline, orphenadrine, pericyazine, phenprocoumon, pipothiazine, promethazine, salicylic acid, theophylline, thiopropazate, trimeprazine, trimipramine
Interfering: butaperazine, imipramine, pipamperone, quinidine, thiothixene

REFERENCE
Edelbroek,P.M.; de Haas,E.J.M.; de Wolff,F.A. Liquid-chromatographic determination of amitryptyline and its metabolites in serum, with adsorption onto glass minimized, *Clin.Chem.*, **1982**, *28*, 2143–2148.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 4.3

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cy-

clizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4 5 μm LiChrospher 100 RP-8
Mobile phase: MeCN:0.025% phosphoric acid:buffer 60:25:15 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 9.45

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxy-

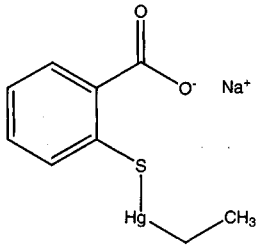
chloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Thimerosal

Molecular formula: $C_9H_9HgNaO_2S$
Molecular weight: 404.82
CAS Registry No.: 54-64-8
Merck Index: 9451



SAMPLE
Matrix: formulations
Sample preparation: Add IS to vaccine at a concentration of 40 μg/mL, centrifuge at 3400 g for 15 min, inject a 25 μL aliquot of the supernatant. (IS stock solution was prepared in mobile phase:water 1:4.)

HPLC VARIABLES
Guard column: 5 × 4 5 μm Hypersil C18
Column: 210 × 4.6 5 μm Hypersil C18
Mobile phase: MeOH:water:orthophosphoric acid 35:35:0.9, pH 2.5
Flow rate: 0.6
Injection volume: 25
Detector: UV 222

CHROMATOGRAM
Retention time: 10.1
Internal standard: salicylic acid (6.5)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
vaccine

REFERENCE
Tleugabulova,D.; Gonzalez Perez,I. Reversed-phase high-performance liquid chromatographic study of thimerosal stability in Cuban recombinant hepatitis B vaccine, *J.Chromatogr.A*, **1996**, *729*, 219–227.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 300 × 3.9 10 μm LiChrosorb Si-60
Mobile phase: MeOH:water 60:40 containing 4 mM disodium citrate and 4 mM tetrabutylammonium bromide, pH 6.0
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: benzalkonium chloride, domiphen bromide, xylometazoline
Interfering: inorganic salts

KEY WORDS
nasal drops

REFERENCE
Lingeman,H.; van Munster,H.A.; Beynen,J.H.; Underberg,W.J.; Hulshoff,A. High-performance liquid chromatographic analysis of basic compounds on non-modified silica gel and aluminium oxide with aqueous solvent mixtures, *J.Chromatogr.*, **1986**, *352*, 261–274.

SAMPLE
Matrix: formulations
Sample preparation: 9.5 mL Contact lens solution + 0.5 mL 3 mg/mL methylparaben, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 7 μm Nucleosil C18 pre-column
Column: 7 μm Nucleosil C18
Mobile phase: MeOH:100 mM KH_2PO_4 adjusted to pH 3.5 with phosphoric acid 60:40
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 11.4
Internal standard: methyl paraben (7.8)
Limit of detection: 100 ng

OTHER SUBSTANCES
Simultaneous: chlorhexidine gluconate, thiosalicylic acid

KEY WORDS
stability-indicating; contact lens solutions

REFERENCE
Hu,O.Y.-P.; Wang,S.-Y.; Fang,Y.-J.; Chen,Y.-H.; King,M.-L. Simultaneous determination of thimerosal and chlorhexidine in solutions for soft contact lenses and its applications in stability studies, *J.Chromatogr.*, **1990**, *523*, 321–326.

SAMPLE
Matrix: formulations
Sample preparation: Inject directly or extract as follows. Condition a Sep-Pak C18 SPE cartridge with MeOH and water. 10 mL Ophthalmic solution + 100 μL concentrated phosphoric acid, add to the SPE cartridge, wash with 5 mL water, wash with 2 mL MeOH:water 20:80, elute with 2 mL MeOH, dilute the eluate to 10 mL with MeOH, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm Spherisorb C18
Mobile phase: MeOH:water 50:50 containing 2 mM tetraethylammonium perchlorate, adjusted to pH 4.8 with perchloric acid
Flow rate: 1
Injection volume: 20
Detector: E, Metrohm Model 461, Metrohm Model 656 flow cell, carbon paste electrode, 0.9 V

CHROMATOGRAM
Retention time: 5
Limit of detection: 90 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products, 2,2'-dithiodibenzoic acid, thiosalicylic acid

KEY WORDS
ophthalmic solutions; SPE

REFERENCE
del Pilar da Silva,M.; Procopio,J.R.; Hernández,L. Evaluation of the capability of different chromatographic systems for the monitoring of thimerosal and its degradation products by high-performance liquid chromatography with amperometric detection, *J.Chromatogr.A*, **1993**, *653*, 267–273.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 10 μm Spherisorb 10 ODS
Mobile phase: MeOH:water:phosphoric acid 60:50:1
Flow rate: 2.6
Injection volume: 25
Detector: UV 222

CHROMATOGRAM
Retention time: 3
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products, 2,2'-dithiosalicylic acid, thiosalicylic acid

REFERENCE
Reader,M.J.; Lines,C.B. Decomposition of thimerosal in aqueous solution and its determination by high-performance liquid chromatography, *J.Pharm.Sci.*, **1983**, *72*, 1406–1409.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of the ophthalmic solution directly.

HPLC VARIABLES
Column: two 250 × 4 10 μm Spherisorb ODS 10 columns in series
Mobile phase: MeOH:water:phosphoric acid 60:50:1
Flow rate: 4
Injection volume: 50

Detector: UV 222

KEY WORDS
ophthalmic solutions

REFERENCE
Reader,M.J. Influence of isotonic agents on the stability of thimerosal in ophthalmic formulations, *J.Pharm.Sci.*, **1984**, *73*, 840–841.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 210 × 4.6 5 μm Spherisorb RP-18
Mobile phase: MeOH:water:phosphoric acid 65:35:0.9
Flow rate: 0.6
Injection volume: 20
Detector: UV 222

CHROMATOGRAM
Retention time: 7.6
Limit of quantitation: 5 ppm

OTHER SUBSTANCES
Simultaneous: degradation products, dithiosalicylic acid, thiosalicylic acid

REFERENCE
Caraballo,I.; Rabasco,A.M.; Fernández-Arévalo,M. Study of thimerosal degradation mechanism, *Int.J.Pharm.*, **1993**, *89*, 213–221.

SAMPLE
Matrix: urine
Sample preparation: Add 0.05% thymol to urine, filter, dilute 1:10 with water, inject an aliquot.

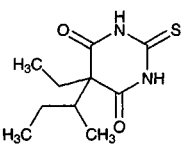
HPLC VARIABLES
Guard column: Guard-Pak C18
Column: 300 × 3.9 μBondapak C18
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 33

OTHER SUBSTANCES
Simultaneous: creatinine, thymol, uric acid

REFERENCE
Chen,Y.; Pietrzyk,R.A.; Whitson,P.A. Quantification of urinary uric acid in the presence of thymol and thimerosal by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *763*, 187–192.

Thiobutabarbital



Molecular formula: $C_{10}H_{16}N_2O_2S$, $C_{10}H_{15}N_2NaO_2S$
Molecular weight: 228.32
CAS Registry No.: 2095-57-0, 947-08-0 (sodium salt)
Merck Index: 9457

SAMPLE
Matrix: solutions

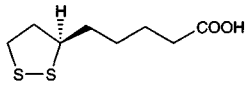
HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChroCART LiChrospher 60 RP Select B
Column: 125 × 4 5 μm LiChroCART LiChrospher 60 RP Select B
Mobile phase: MeCN:buffer 50:50 (Buffer was 25 mM pH 3.0 triethylammonium phosphate containing 2% MeCN.)
Flow rate: 2
Injection volume: 50
Detector: UV 283

CHROMATOGRAM
Retention time: 2.70

OTHER SUBSTANCES
Simultaneous: thiopental

REFERENCE
Hannak,D.; Scharbert,F.; Kattermann,R. Stepwise binary gradient high-performance liquid chromatographic system for routine drug monitoring, *J.Chromatogr.A*, **1996**, *728*, 307–310.

Thioctic acid



Molecular formula: $C_8H_{14}O_2S_2$
Molecular weight: 206.33
CAS Registry No.: 62-46-4, 1200-22-2 (d-form), 1077-28-7 (dl-form), 1077-27-6 (l-form), 2319-84-8 (sodium salt)
Merck Index: 9462

SAMPLE
Matrix: solutions
Sample preparation: Mix 200 μL of a solution in MeOH:water 10:90 with 150 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer and 100 μL 4.2 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, sonicate for 3 min, let stand for 22 min, add 150 μL 4.5 μg/mL IS in MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES
Column: 250 × 4.5 Hypersil 5 ODS
Mobile phase: MeCN:MeOH:water 37.4:30.6:32
Column temperature: 35
Flow rate: 1.1
Injection volume: 50
Detector: F ex 300 em 460

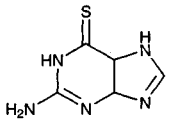
CHROMATOGRAM
Retention time: 16.5
Internal standard: n-hexanoic acid 6-methoxynaphthacyl ester (?) (Dissolve 2 mmole n-hexanoic acid and 1 mmole 2-bromoacetyl-6-methoxynaphthalene in 10 mL anhydrous MeCN, add 500 μL triethylamine, heat to 60° for 30 min, cool, add 30 mL water, extract three times with 10 mL portions of diethyl ether. Combine the organic layers and wash them with 5% sodium bicarbonate solution, wash three times with 10 mL portions of water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from MeOH/water

to give 6-methoxynaphthacyl ester of n-hexanoic acid (mp 79-80°) (J. Pharm. Biomed. Anal. 1993, 11, 761) (19)

KEY WORDS
derivatization

REFERENCE
Gatti,R.; Bousquet,E.; Bonazzi,D.; Cavrini,V. Determination of carboxylic acid salts in pharmaceuticals by high-performance liquid chromatography after pre-column fluorogenic labelling, *Biomed.Chromatogr.*, **1996**, *10*, 19–24.

Thioguanine



Molecular formula: $C_5H_5N_5S$
Molecular weight: 167.19
CAS Registry No.: 154-42-7, 5580-03-0 (hemihydrate)
Merck Index: 9473
Lednicer No.: 2 464

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1 mL Plasma + 1 mL cold 2 M perchloric acid, mix, centrifuge at 4° at 48000 g for 20 min. Remove a 1 mL aliquot of the supernatant and adjust to pH 10-12 with 150 μL 4 M KOH, let stand at 4° for 2 days. Adjust the pH of the supernatant to 2-3 with 150 μL 1 M HCl, centrifuge at 700 g for 10 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:buffer 10:90 (Buffer was 10 mM acetic acid adjusted to pH 3.5 with 5 M NaOH. Flush column daily with MeOH:water 50:50.)
Flow rate: 2
Injection volume: 100
Detector: UV 340

CHROMATOGRAM
Retention time: 4
Limit of detection: 800 nM

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Andrews,P.A.; Egorin,M.J.; May,M.E.; Bachur,N.R. Reversed-phase high-performance liquid chromatography analysis of 6-thioguanine applicable to pharmacologic studies in humans, *J.Chromatogr.*, **1982**, *227*, 83–91.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 400 mM NaOH + 1 mL 0.3% phenylmercuric acetate in ethyl acetate + 3 mL diethyl ether, shake on a tumble mixer for 10 min, centrifuge for 5 min. Remove the organic layer and add it to 500 μL 100 mM HCl, whirlmix for 2 min, centrifuge for 5 min, discard the organic layer, evaporate traces of organic solvent under a stream of nitrogen at room temperature for 15 min, add 10 μL 3 mg/mL dithioerythritol in water, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 LiChrosorb 10 RP-18
Mobile phase: Isopropanol:water 3:97 containing 13.80 g/L $NaH_2PO_4.H_2O$, 200 μL/L 85% phosphoric acid, 60 mg/L dithioerythritol, and 500 mg/L sodium octanesulfonate, pH 3.6-3.7
Flow rate: 1.5
Detector: F ex 295 em 380 following post-column reaction. The column effluent mixed with 8 mM potassium chromate in 500 mM HCl pumped at 0.16 mL/min and with air flowing at 0.32 mL/min and the mixture flowed through a single mixing coil. The effluent from this coil mixed with 1.6% sodium metabisulfite pumped at 0.16 mL/min and this mixture flowed through a single mixing coil. The effluent from this coil mixed with 4 M ammonium hydroxide pumped at 0.23 mL/min and this mixture flowed through a double mixing coil to a debubbler. The liquid effluent from the debubbler flowed to the detector.

CHROMATOGRAM

Retention time: 8
Internal standard: 6-thioguanine

OTHER SUBSTANCES

Extracted: 6-mercaptopurine

KEY WORDS

post-column reaction; plasma; 6-thioguanine is IS

REFERENCE

Jonkers,R.E.; Oosterhuis,B.; ten Berge,R.J.M.; van Boxtel,C.J. Analysis of 6-mercaptopurine in human plasma with a high-performance liquid chromatographic method including post-column derivatization and fluorimetric detection, *J.Chromatogr.*, **1982**, *233*, 249–255.

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 80 μL water + 10 μL 1 M dithiothreitol, vortex for 10 s, add 2 mL MeCN, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the supernatant and add it to 2 mL dichloromethane, shake on a reciprocating shaker for 5 min, centrifuge at 2000 g for 5 min. Remove 750 μL from the top aqueous layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 150 μL distilled water, vortex for 1 min, inject a 15 μL aliquot.

HPLC VARIABLES

Guard column: 70 × 2.2 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:acetic acid:water 3.5:0.2:96.3
Flow rate: 1.4
Injection volume: 15
Detector: UV 322

CHROMATOGRAM

Retention time: 6.5
Internal standard: 6-thioguanine

OTHER SUBSTANCES

Extracted: 6-mercaptopurine
Noninterfering: caffeine, cytarabine, 5-fluorouracil, prednisone, theophylline, vinblastine, vincristine

KEY WORDS

plasma; protect from light; monkey; human; 6-thioguanine is IS

REFERENCE

Narang,P.K.; Yeager,R.L.; Chatterji,D.C. Quantitation of 6-mercaptopurine in biologic fluids using high-performance liquid chromatography: a selective and novel procedure, *J.Chromatogr.*, **1982**, *230*, 373–380.

SAMPLE
Matrix: blood
Sample preparation: Dilute 10 mL blood with 15 mL phosphate buffered saline containing 3 mM ethyleneglycoltetraacetic acid. Layer 8 mL diluted blood on 2 mL Ficoll-Hypaque (Sigma), centrifuge at 9000 g for 10 min, collect lymphocyte band, wash with PBS. 200 μL Lymphocytes + 50 μL 1 M sulfuric acid, heat at 100° for 45 min, cool, centrifuge. Remove 200 μL and add to 55 μL 1 M pH 10.1 sodium bicarbonate, add 1.5 mL ethyl acetate:dichloromethane containing 100 ng/mL sulfamethoxazole, vortex, centrifuge. Remove 175 μL of the upper aqueous layer and add it to 25 μL 1 M pH 10.1 sodium bicarbonate, add 25 μL 0.5% potassium permanganate (freshly prepared), let stand for 5 min, add 5 μL 15% hydrogen peroxide, vortex, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:buffer 11:89 (Buffer was 10 mM pH 7 sodium phosphate containing 0.06% tetrabutylammonium chloride.)
Column temperature: 45
Flow rate: 1.5
Injection volume: 10
Detector: F ex 330 em 412 (370 nm cut-off filter)

CHROMATOGRAM
Retention time: 2.30
Limit of detection: 1 ng

KEY WORDS
whole blood; lymphocytes

REFERENCE
Erdmann,G.R.; Steury,J.C.; Carleton,B.C.; Stafford,R.J.; Bostrom,B.C.; Canafax,D.M. Reversed-phase high-performance liquid chromatographic approach to determine total lymphocyte concentrations of 6-thioguanine, methylmercaptopurine and methylthioguanine in humans, *J.Chromatogr.*, **1991**, *571*, 149–156.

SAMPLE
Matrix: blood
Sample preparation: 250 μL Plasma + 25 μL 1 M aluminum perchlorate in water, let stand at room temperature for 15 min, chill in ice water for 15 min, centrifuge at 15600 g for 15 min, discard the supernatant. Suspend the precipitate in 500 μL 50 mM aluminum perchlorate in water by stirring to break up the precipitate, vortex for 20 s, centrifuge at 15600 g for 15 min, discard the supernatant. Suspend the precipitate in 150 μL 400 mM perchloric acid, add 5 μL freshly prepared 200 mM aqueous sodium hydrosulfite, mix, let stand at room temperature for 30 min, chill in ice water, centrifuge at 15600 g for 15 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 45 × 2 40 μm ODS
Column: 150 × 4.3 5 μm Ultrasphere ODS
Mobile phase: Water:85% phosphoric acid 99.32:0.68 containing 154.3 mg/L dithiothreitol
Flow rate: 1
Injection volume: 50
Detector: UV 340

CHROMATOGRAM
Retention time: 4.73
Internal standard: 6-thioguanine
Limit of quantitation: 3 ng/mL

OTHER SUBSTANCES
Extracted: 6-mercaptopurine, 6-thiouric acid

KEY WORDS
plasma; pharmacokinetics; 6-thioguanine is IS

REFERENCE

Lin,K.T.; Varin,F.; Rivard,G.E.; Leclerc,J.M. Isolation of 6-mercaptopurine in human plasma by aluminum ion complexation for high-performance liquid chromatographic analysis, *J.Chromatogr.*, **1991**, *536*, 349–355.

SAMPLE

Matrix: blood

Sample preparation: Add 1 volume ice-cold 8 M perchloric acid to 20 volumes plasma, mix, keep on ice for 10 min, centrifuge at 10 000 g for 15 min, remove the supernatant. Adjust the pH of the supernatant to 6-7 with 10 volumes ice-cold 4 M K_2KHPO_4, keep on ice for 10 min, centrifuge at 10 000 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-18-DB

Mobile phase: Gradient. A was 25 mM KH_2PO_4. B was MeOH:50 mM KH_2PO_4 25:75. A:B from 98:2 to 96:4 over 5 min, to 85:15 over 3 min, to 80:20 over 2 min, to 40:60 over 10 min, maintain at 40:60 over 2 min, to 20:80 over 3 min, maintain at 20:80 for 20 min, return to initial conditions over 3 min, re-equilibrate for 12 min.

Flow rate: 1.25

Injection volume: 100

Detector: UV 342

CHROMATOGRAM

Retention time: 10

Limit of detection: 20-50 nM

OTHER SUBSTANCES

Extracted: metabolites, mercaptopurine (UV 320)

KEY WORDS

plasma

REFERENCE

Keuzenkamp-Jansen,S.W.; De Abreu,R.A.; Bökkerink,J.P.M.; Trijbels,J.M.F. Determination of extracellular and intracellular thiopurines and methylthiopurines by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *672*, 53–61.

SAMPLE

Matrix: blood, CSF, urine

Sample preparation: Blood, CSF. Collect 2 mL blood in tubes containing heparin and 120 μg dithiothreitol (DTT). Mix, centrifuge at 2000 g for 5 min, remove plasma. CSF. Collect 0.5 mL CSF in tubes containing 30 μg DTT. Cool CSF and plasma samples on ice, add freshly prepared ice-cold 50% trichloroacetic acid equal to 10% of sample volume. Urine. Collect 2 mL urine in tubes containing 120 μg DTT, filter (0.22 μm), inject an aliquot.

HPLC VARIABLES

Column: two 250 × 4.6 10μm Nucleosil 10 C18 columns in series

Mobile phase: Gradient. A was 25 mM pH 2.75 phosphoric acid. B was MeOH:water 50:50. C was 100 mM pH 6.6 KH_2PO_4. A:B:C from 100:0:0 to 98:2:0 over 5 min, to 30:3.5:66.5 over 5 min, maintain at 30:3.5:66.5 for 10 min, re-equilibrate at initial conditions for 10 min.

Column temperature: 33

Flow rate: 1.7

Injection volume: 195, 500

Detector: UV 342

CHROMATOGRAM

Retention time: 13

Limit of detection: 25 nM

OTHER SUBSTANCES

Extracted: metabolites, mercaptopurine (UV 312), 6-mercaptopurine riboside, 6-thioguanosine

KEY WORDS
plasma; goat; pharmacokinetics

REFERENCE
van Baal,J.M.; van Leeuwen,M.B.; Schouten,T.J.; De Abreu,R.A. Sensitive high-performance liquid chromatographic determination of 6-mercaptopurine, 6-thioguanine, 6-mercaptopurine riboside and 6-thioguanosine in biological fluids, *J.Chromatogr.*, **1984**, *336*, 422–428.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Filter (Amicon Model MM 302, type PM 10 Diaflo membrane) plasma while centrifuging at 8000 g for 3 min, inject a 20-100 μL aliquot of the ultrafiltrate. Urine. Inject an aliquot directly.

HPLC VARIABLES
Column: 150 × 4 Nucleosil 5C8
Mobile phase: 50 mM pH 7.0 citrate-phosphate buffer (Buffer was 9.61 g $Na_2HPO_4.H_2O$ and 1.33 g citric acid monohydrate in 1 L water, pH 7.0.)
Flow rate: 1.4
Injection volume: 20-100
Detector: UV 343

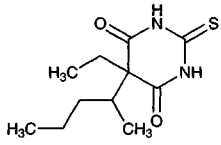
CHROMATOGRAM
Retention time: 5.7
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: 6-mercaptopurine
Simultaneous: allopurinol, azaguanine, guanine, oxipurinol, uric acid
Noninterfering: adenine, 2-amino-6-methylthiopurine, aspirin, benzbromarone, caffeine, diazepam, dihydralazine, dipyridamole, fluorouracil, hypoxanthine, methotrexate, procarbazine, propranolol, spironolactone, sulfamethoxazole, sulfinpyrazone, theophylline, thiouric acid, thioxanthine, trimethoprim, xanthine

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Breithaupt,H.; Goebel,G. Quantitative high pressure liquid chromatography of 6-thioguanine in biological fluids, *J.Chromatogr.Sci.*, **1981**, *19*, 496–499.

Thiopental



Molecular formula: $C_{11}H_{18}N_2O_2S$
Molecular weight: 242.34
CAS Registry No.: 76-75-5, 71-73-8 (Na salt)
Merck Index: 9487
Lednicer No.: 1 274

SAMPLE
Matrix: blood
Sample preparation: Precipitate 100 μL serum with 200 μL 10 μg/mL IS in MeCN, centrifuge at 12000 g for 5 min. Inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChroCART LiChrospher 60 RP Select B